

CLAIMS

1. An isolated nucleic acid molecule comprising a polynucleotide having a sequence encoding a peptide or polypeptide of Smac having at least two contiguous amino acid residues derived from at least residues 56-139 of SEQ ID NO:1 and of which up to 184 contiguous amino acid residues can be derived from residues 56-239 of SEQ ID NO:1, a functional variant of each or a functional equivalent of each, each of which is capable of specifically binding to at least a portion of an Inhibitor of Apoptosis protein.
2. The isolated nucleic acid molecule of claim 1, wherein said portion is at least one BIR domain.
3. The isolated nucleic acid molecule of claim 2, wherein said BIR domain is BIR1.
4. The isolated nucleic acid molecule of claim 2, wherein said BIR domain is BIR2.
5. The isolated nucleic acid molecule of claim 2, wherein said BIR domain is BIR3.
6. The isolated nucleic acid molecule of claim 1, wherein said specific binding is to a full-length IAP
7. The isolated nucleic acid molecule of claim 1, wherein said peptide or polypeptide has an amino acid sequence of at least Ala-Val
8. The isolated nucleic acid molecule of claim 1, wherein said peptide or polypeptide has an amino acid sequence of at least the sequence provided in SEQ ID NO:13.

9. An isolated nucleic acid molecule consisting essentially of a polynucleotide having a sequence encoding a peptide or polypeptide of Smac having at least two contiguous amino acid residues derived from at least residues 56-139 of SEQ ID NO:1 and of which up to 184 contiguous amino acid residues can be derived from residues 56-239 of SEQ ID NO:1, a functional variant of each or a functional equivalent of each, each of which is capable of specifically binding to at least a portion of an Inhibitor of Apoptosis protein.

10. The isolated nucleic acid molecule of claim 9, wherein said portion is at least one BIR domain.

11. The isolated nucleic acid molecule of claim 10, wherein said BIR domain is BIR1.

12. The isolated nucleic acid molecule of claim 10, wherein said BIR domain is BIR2.

13. The isolated nucleic acid molecule of claim 10, wherein said BIR domain is BIR3.

14. The isolated nucleic acid molecule of claim 9, wherein said specific binding is to a full-length IAP

15. The isolated nucleic acid molecule of claim 9, wherein said peptide or polypeptide has an amino acid sequence of at least Ala-Val.

16. The isolated nucleic acid molecule of claim 9, wherein said peptide or polypeptide has an amino acid sequence of at least the sequence provided in SEQ ID NO:13.

17. An isolated nucleic acid molecule consisting of a polynucleotide having a sequence encoding a peptide or polypeptide of Smac having at least two contiguous amino acid residues derived from at least residues 56-139 of SEQ ID NO:1 and of which up to 184 contiguous amino acid residues can be derived from residues 56-239 of SEQ ID NO:1, a functional variant of each or a functional equivalent of each, each of which is capable of specifically binding to at least a portion of an Inhibitor of Apoptosis protein.

18. The isolated nucleic acid molecule of claim 17, wherein said portion is at least one BIR domain.

19. The isolated nucleic acid molecule of claim 18, wherein said BIR domain is BIR1.

20. The isolated nucleic acid molecule of claim 18, wherein said BIR domain is BIR2.

21. The isolated nucleic acid molecule of claim 18, wherein said BIR domain is BIR3.

22. The isolated nucleic acid molecule of claim 17, wherein said specific binding is to a full-length IAP

23. The isolated nucleic acid molecule of claim 17, wherein said peptide or polypeptide has an amino acid sequence of at least Ala-Val.

24. The isolated nucleic acid molecule of claim 17, wherein said peptide or polypeptide has an amino acid sequence of at least the sequence provided in SEQ ID NO:13.

25. An expression vector comprising a nucleic acid molecule of claim 1 operatively linked to regulatory elements.

26. The expression vector of claim 25, wherein the regulatory elements include an inducible promoter.

27. A host cell transformed with an expression vector of claim 25.

28. An isolated peptide or polypeptide comprising an amino acid sequence having at least two contiguous amino acid residues derived from at least residues 56-139 of SEQ ID NO:1 and of which up to 184 contiguous amino acid residues can be derived from residues 56-239 of SEQ ID NO:1, a functional variant of each or a functional equivalent of each, each of which is capable of specifically binding to at least a portion of an Inhibitor of Apoptosis protein.

29. The isolated peptide or polypeptide of claim 28, wherein said portion is at least one BIR domain.

30. The isolated peptide or polypeptide of claim 29, wherein said BIR domain is BIR1.

31. The isolated peptide or polypeptide of claim 29, wherein said BIR domain is BIR2.

32. The isolated peptide or polypeptide of claim 29, wherein said BIR domain is BIR3.

33. The isolated peptide or polypeptide of claim 28, wherein said specific binding is to a full-length IAP

34. The isolated peptide or polypeptide of claim 28, wherein said peptide or polypeptide has an amino acid sequence of at least Ala-Val.

35. The isolated peptide or polypeptide of claim 28, wherein said peptide or polypeptide has an amino acid sequence of at least the sequence provided in SEQ ID NO:13.

36. An isolated Smac peptide or polypeptide consisting essentially of an amino acid sequence having at least two contiguous amino acid residues derived from at least residues 56-139 of SEQ ID NO:1 and of which up to 184 contiguous amino acid residues can be derived from residues 56-239 of SEQ ID NO:1, a functional variant of each or a functional equivalent of each, each of which is capable of specifically binding to at least a portion of an Inhibitor of Apoptosis protein.

37. The isolated peptide or polypeptide of claim 36, wherein said portion is at least one BIR domain.

38. The isolated peptide or polypeptide of claim 37, wherein said BIR domain is BIR1.

39. The isolated peptide or polypeptide of claim 37, wherein said BIR domain is BIR2.

40. The isolated peptide or polypeptide of claim 37, wherein said BIR domain is BIR3.

41. The isolated peptide or polypeptide of claim 36, wherein said specific binding is to a full-length IAP

42. The isolated peptide or polypeptide of claim 36, wherein said peptide or polypeptide has an amino acid sequence of at least Ala-Val.

43. The isolated peptide or polypeptide of claim 36, wherein said peptide or polypeptide has an amino acid sequence of at least the sequence provided in SEQ ID NO:13.

44. An isolated Smad peptide or polypeptide consisting of an amino acid sequence having at least two contiguous amino acid residues derived from at least residues 56-139 of SEQ ID NO:1 and of which up to 184 contiguous amino acid residues can be derived from residues 56-239 of SEQ ID NO:1, a functional variant of each or a functional equivalent of each, each of which is capable of specifically binding to at least a portion of an Inhibitor of Apoptosis protein.

45. The isolated peptide or polypeptide of claim 44, wherein said portion is at least one BIR domain.

46. The isolated peptide or polypeptide of claim 45, wherein said BIR domain is BIR1.

47. The isolated peptide or polypeptide of claim 45, wherein said BIR domain is BIR2.

48. The isolated peptide or polypeptide of claim 45, wherein said BIR domain is BIR3.

49. The isolated peptide or polypeptide of claim 44, wherein said specific binding is to a full-length IAP

50. The isolated peptide or polypeptide of claim 44, wherein said peptide or polypeptide has an amino acid sequence of at least Ala-Val.

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51. The isolated peptide or polypeptide of claim 44, wherein said peptide or polypeptide has an amino acid sequence of at least the sequence provided in SEQ ID NO:13.

52. A method for inducing apoptosis in a cell, comprising contacting the cell with at least one component selected from the group consisting of:

(a) a peptide or polypeptide of claim 28 and

(b) a nucleic acid molecule one of claim 1,

under conditions and for a time sufficient to permit the induction of apoptosis in the cell.

53. A method of stimulating apoptosis in a neoplastic or tumor cell, comprising contacting the cell with at least one component selected from the group consisting of:

(a) a peptide or polypeptide of claim 28 and

(b) a nucleic acid molecule of claim 1,

under conditions and for a time sufficient to permit the induction of apoptosis in the cell.

54. The method of claim 53, wherein said cell overexpresses an inhibitor of a caspase.

55. The method of claim 54, wherein the inhibitor inhibits activation or activity of a caspase selected from the group consisting caspase-3, caspase-7 and caspase -9.

56. The method of claim 55, wherein the inhibitor is at least a portion of an Inhibitor of Apoptosis protein.

57. A method of identifying an inhibitor or enhancer of a caspase-mediated apoptosis comprising:

(a) contacting a cell transformed or transfected with a vector expressing the peptide or polypeptide of claim 28 with a candidate inhibitor or candidate enhancer; and

(b) detecting cell viability,

wherein an increase in cell viability indicates the presence of an inhibitor and a decrease in cell viability indicates the presence of an enhancer.

58. A method of identifying an inhibitor or enhancer of a caspase-mediated apoptosis comprising:

(a) contacting a cell transformed or transfected with a vector expressing the peptide or polypeptide of claim 28 with a candidate inhibitor or candidate enhancer; and

(b) detecting the presence of large and small caspase subunits, and therefrom determining the level of caspase processing activity,

wherein a decrease in processing indicates the presence of an inhibitor and an increase in processing indicates the presence of an enhancer.

59. The method of claim 58, wherein the caspase detected is selected from the group consisting of caspase-3, caspase-7 and caspase-9.

60. A method for identifying a compound that inhibits apoptosis comprising:

- (a) separately contacting a plurality of cell populations expressing a cytosolic Smac and an inhibitor of BID with a compound to be tested for apoptotic inhibiting activity;
- (b) incubating said cell populations with a direct stimulus of the cell death pathway; and
- (c) measuring the specific apoptotic activity of the cell populations, wherein inhibition of the specific apoptotic activity is indicative that said compound is an inhibitor of apoptosis.

61. The method of claim 60, wherein said direct stimulus of the cell death pathway is selected from the group consisting of Fas ligand, anti-Fas antibody and staurosporine UV and gamma irradiation.

62. The method of claim 60, wherein (c) further comprises lysing said cells and determining caspase activity in said lysate.

63. The method of claim 60, wherein said compound exhibits caspase inhibitory activity.

64. The method of claim 60, wherein said compound inhibits apoptosis by promoting the activity of a cell survival polypeptide.

65. The method of claim 60, wherein said compound exhibits cell death polypeptide inhibitory activity.

66. A method for identifying a compound that inhibits Smac binding to a Smac-binding molecule, comprising:

(a) contacting a candidate compound with a Smac peptide in the presence of a Smac-binding molecule; and

(b) detecting displacement or inhibition of binding of said Smac-binding molecule from said Smac peptide.

67. The method of claim 66, wherein the Smac-binding molecule is at least a portion of an IAP.

68. The method of claim 67, wherein said portion is at least one BIR domain.

69. The method of claim 68, wherein said BIR domain is BIR1.

70. The method of claim 68, wherein said BIR domain is BIR2.

71. The method of claim 68, wherein said BIR domain is BIR3.

72. The method of claim 67, wherein the Smac-binding molecule is a full length IAP.

73. A method for identifying a compound that inhibits Smac from being to a Smac-binding molecule, comprising:

(a) contacting a candidate compound with a Smac peptide in the presence of a Smac-binding molecule; and

(b) performing a functional assay that confirms displacement of said Smac-binding molecule from said Smac peptide.

74. The method of claim 73, wherein the functional assay detects the presence of large and small caspase subunits, and therefrom determining the level of caspase processing activity, wherein a decrease in processing confirms displacement.

75. The method of claim 74, wherein the caspase detected is selected from the group consisting of caspase-3, caspase-7 and caspase-9.

76. The method of claim 75, wherein the functional assay detects the presence of a substrate cleavage product produced by a caspase cleavage of a substrate.

77. The method of claim 76, wherein said substrate is acetyl DEVD-aminomethyl coumarin.

78. An antibody that specifically binds to a peptide or polypeptide of claim 28.

79. An antibody that specifically binds to an epitope located on the N-terminus of Smac.

80. The antibody of claim 79, wherein said antibody inhibits the binding of Smac to at least a portion of an IPA.

81. The antibody of claim 80, wherein said portion is at least one BIR domain.

82. The antibody of claim 81, wherein said BIR domain is BIR1.

83. The antibody of claim 81, wherein said BIR domain is BIR2.

84. The antibody of claim 81, wherein said BIR domain is BIR3.

85. The antibody of claim 80, wherein said antibody inhibits the binding to a full-length IAP.

86. The antibody of claim 78, wherein said antibody binds to an epitope that includes the amino acid sequence provided in SEQ ID NO:13.

87. A composition comprising a nucleic acid molecule of the claim 1, and a physiologically acceptable carrier.

88. A composition comprising a peptide of claim 28, and a physiologically acceptable carrier.

89. A composition comprising an antibody of claim 78, and a physiologically acceptable carrier.

90. A composition comprising an antibody of claim 79, and a physiologically acceptable carrier.

91. An isolated nucleic acid molecule comprising a polynucleotide having a sequence encoding a cytosolic isoform of Smac.

92. An isolated nucleic acid molecule consisting essentially of a polynucleotide having a sequence encoding a cytosolic isoform Smac.

93. An isolated nucleic acid molecule consisting of a polynucleotide having a sequence encoding a cytosolic isoform of Smac.

94. An isolated polypeptide comprising an amino acid sequence for a cytosolic isoform of Smac.

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96. An isolated polypeptide consisting of an amino acid sequence for a cytosolic isoform.

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